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PeiRu Dai

494997473@qq.com

Yichun University

Yan Chen

Yichun University

YinYin Wei

Yichun University

WeiHua Yin

The People's Hospital of Yichun Affiliated to Clinical Medicine School

ChunLin Chen

Yichun University

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Genipin as the Active Compound of Gardenia fruits Inhibit Proliferation, Migration and Autophagy of Prostate Cancer through PI3K/AKT Signaling Pathway

PeiRu Dai^{1*}, Yan Chen^{1†}, YinYin Wei^{1†}, WeiHua Yin^{2†}, ChunLin Chen^{1†}

^{1*}School of Chemistry and Biological Engineering , Yichun University , Yichun, 336000, JiangXi, China.

²Department of Oncology , The People's Hospital of Yichun Affiliated to Clinical Medicine School , Yichun, 336000, JiangXi, China.

*Corresponding author(s). E-mail(s): 494997473@qq.com;

Contributing authors: chenyan_023@163.com; wyy10201@outlook.com; [ywh1939@163](mailto:ywh1939@163.com) ; 31757847@qq.com;

[†]These authors contributed equally to this work.

Abstract

Purpose: Prostate cancer is the second most diagnosed cancer type and the most common cause of cancer-related deaths in men. Genipin is a protein cross-linking agent extracted from Gardenia (*Gardenia jasminoides* Ellis) fruits, which has been reported to exhibit antitumor activity against several types of cancer. The aim of the present study was to investigate the antitumor effect of Genipin on prostate cancer and the underlying molecular mechanisms.

Methods: CCK-8 assays were performed to determine the cell viability of genipin prostate cancer cells lines (DU145, PC-3, RM-1) and normal prostate cell lines (WPMY1). Colony formation assays, and wound-healing assays used to detect the effect of genipin on DU145, PC-3 and RM-1 cell lines. Immunofluorescence and western blotting were carried out to investigate its mechanism.

Results: Genipin inhibited the survival of DU-145, PC-3 and RM-1 cells in a time-dependent and dose-dependent manner. And Genipin induced apoptosis of prostate carcinoma cells, including DU-145, PC-3 and RM-1 cells. Moreover, the expression of p62 in DU-145 and PC-3 cells was down-regulated, whereas Beclin1 and LC3II/ I were up-regulated. In addition, genipin also decreased p-PI3K, p-AKT, p-NF- κ B and p-mTOR.

Conclusion: Genipin is the active compound to inhibit proliferation and migration of prostate cancer through PI3K/AKT/NF- κ B signaling pathway and promoted prostate cancer cells autophagy through PI3K/AKT/mTOR signaling pathway, indicating that Genipin may serve as a potential lead drug for prostate cancer treatment. Therefore, Genipin may be used as a novel therapeutic agent in the treatment of prostate cancer.

Keywords: Genipin, Prostate cancer, PI3K/AKT/NF- κ B, PI3K/AKT/mTOR

1 Introduction

Prostate cancer continues to pose a major challenge to male health around the world. The incidence and death rate of prostate cancer is reported to be the second highest among men [Siegel et al \(2023\)](#) .Current treatment options include AR-targeted agents, chemotherapy, radiotherapy and the surgery [Rebello et al \(2021\)](#). However, Prostate cancer is easy to spread, recurrent, and also resistant to chemotherapy, leading to high mortality [Sartor and de Bono \(2018\)](#). So, it is very important to find effective drugs with low toxicity to treat prostate cancer.

Herbal medicine plays an important role in preventing and curing cancer and other diseases worldwide, particularly in Asia [Tariq et al \(2017\)](#). It has been proved that traditional Chinese medicine(TCM) has many biological activities and clinical effects, especially the monomer of traditional Chinese medicine has the advantages of good therapeutic effect and high safety [Cheung \(2011\)](#). Genipin is a protein cross-linking agent extracted from Gardenia (*Gardenia jasminoides* Ellis) fruits [Xiao et al \(2017\)](#), which has been used as a traditional Chinese medicine over several decades, and recent reports have demonstrated that Genipin possesses medicinal properties, including anti-inflammatory [Huertas-Bello et al \(2023\)](#), anti-angiogenic [Park et al \(2003\)](#) and antidepressant [Araki et al \(2014\)](#). Besides, Genipin has been widely used for drug delivery due to its excellent biocompatibility, admirable biodegradability, and stable cross-linking attributes [Masri et al \(2023\)](#). Genipin has also exhibited significant anti-tumor activity against several types of cancer, such as gastric cancer [Kim et al \(2020\)](#), hepatocellular carcinoma [Tan et al \(2016\)](#), breast cancer [Ayyasamy et al \(2011\)](#) and pancreatic adenocarcinoma [Dando et al \(2017\)](#). However, the effect of Genipin on prostate cancer and its underlying mechanism remain unclear.

Inhibition of the growth and metastasis of cancer cells is an important aspect of anticancer strategies. Cell migration is necessary for cancer metastasis to reach distant locations, often leading to death from cancer [Fonseca Teixeira et al \(2023\)](#). The PI3K/AKT pathway is involved in a variety of biological processes, such as cell growth, survival, metabolism, invasion and migration [Islam et al](#)

[\(2023\)](#). In a large number of patients with prostate cancer, PI3K/AKT signaling is abnormally activated [Islam et al \(2023\)](#). AKT, a serine/threonine kinase, regulates mTOR, NF- κ B, FKHR, Bad, and so on [Fruman and Rommel \(2014\)](#). The transcription factor NF- κ B, trans locates to the nucleus to facilitate the transcription of oncogene after activation in response to a variety of stimuli [Sruthi et al \(2024\)](#). Decreased phosphorylation of PI3K/AKT/NF- κ B has been shown to be associated with progression of prostate cancer [Erdogan et al \(2016\)](#). Mammalian target of rapamycin (mTOR), a serine/threonine kinase, regulate varieties of fundamental cellular processes which include protein synthesis, growth, metabolism, aging, regeneration, autophagy, etc [Murugan \(2019\)](#) . Autophagy plays a crucial role in the survival of cells and associated with PI3K/AKT/mTOR signaling pathway.

In this study, we studied the therapeutic effects of Genipin on prostate cancer cells. In addition, Genipin has been demonstrated to induce mitochondrial apoptosis and to inhibit the migration of prostate carcinoma cells through the PI3K/AKT/NF- κ B pathway and promoted prostate cancer cells autophagy through PI3K/AKT/mTOR signaling pathway. The purpose of this study is to provide a basis for further study on the use of on Genipin in the treatment of prostate cancer.

2 Materials and methods

2.1 Reagents and Antibodies

The chemical compounds Genipin were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China) The Cell Counting Kit-8 (CCK8) was obtained from Dalian Meilun Biotechnology Co., Ltd. (Dalian, Liaoning, China). The Apoptosis and Necrosis Assay kit was supplied by Beyotime Biotech Inc (Shanghai, China) The BCA protein quantitation assay kit was supplied by Shanghai Epizyme Biomedical Technology Co., Ltd (Shanghai, China). The primary antibodies against PI3K, NF- κ B, p- NF- κ B, BAX, Bcl-2 , SURVIVIN, MMP₂ and Caspase-3 were purchased from Wanleibio (Shenyang, China). The PI 3Kinase p85 (phospho Tyr464) antibody was supplied by AiFang biological (Hunan, China). The secondary antibodies

anti-rabbit IgG were obtained from Beijing 4A Biotech (Beijing, China) and antimouse IgG were obtained from TansGen Biotech (Beijing, China). Other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Cell Line and Cell Culture

The human prostate cancer cell line DU145, PC-3 and the mouse prostate cancer cell line RM-1 were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). The human prostate cell line WPMY1 were obtained from FuHeng Biology (Shanghai, China). Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) in 5% CO₂ containing incubator at 37°C.

2.3 Cell Viability Assay

The viability of WPMY1, DU145, PC-3 and RM-1 cells was determined by the Cell Counting Kit-8 (CCK-8) test. Cells (10000/well) were seeded into 96 well plates and cultivated overnight. After 24 hours, 48 hours, 72 hours after treatment with different concentrations of Genipin (10, 30, 50, 75, 100, 200, 300 μ M), the cells were exposed to 100 μ L/well diluted CCK-8 solution. Then, a microplate reader (Bio-Rad Laboratories, Inc., USA) was used for detection of light absorption at 450nm.

2.4 Colony Formation Assay

DU145, PC-3 and RM-1 cells were seeded into a culture dish of 1000 cells per plate and incubated for 2 weeks. Cell culture medium was renewed every 3 days. Two weeks later, the cells were fixed in 4% polyformaldehyde (Changsha Fenlukou Plastic Chemical Plant Business Department) for 15min and stained for 30 minutes on a shaker with Crystal Violet (0.5 mg/mL, Tianjin Institute of Chemical Reagents Co., Ltd). Wash off excess crystal violet. After drying the fixed Crystal Violet was washed with 10% acetic acid (Shanghai Macklin Biochemical Technology Co., Ltd) and then, the absorption of light at 595nm was detected using a microplate reader (Bio-Rad Laboratories, USA).

2.5 Hoechst 33342/PI Staining Assay

DU145, PC-3 and RM-1 cells (2 $\times$ 10⁵/well) were seeded into 6 well plates. After 24 hours of cultivation, the cells were treated with Genipin at 0, 10, 50, and 100 μ M for 24 hours. The cells were then washed with PBS. Then, the cells were stained simultaneously with Hoechst 33342 and PI for 30 minutes at 4°C by a fluorescent microscope (Nikon, Tokyo, Japan). The nuclear morphology of DU145, PC-3 and RM-1 cells were observed.

2.6 Wound-Healing Assay

DU145, PC-3 and RM-1 (5 $\times$ 10⁵/well) were seeded into 6 well plates. When 90% confluency was achieved, the cells were scratched in a straight line using a 200 μ L pipette tip. Then, the different concentration of Genipin (10, 30, 50, 75 μ M) with 1% serum medium were added. An ECLIPSE Ti2-U inverted microscope (Nikon, Tokyo, Japan) was used to capture the images of cells treated at various times (12, 24, 36 hours).

2.7 Western Blot Analysis

After 24 hours of treatment with Genipin, DU145, PC-3 and RM-1 cells were removed with 1ml pipette tip. Then, a RIPA lysis buffer (containing 1 mM PMSF and 1 $\times$ protease inhibitor) was added to obtain the whole cell protein. The BCA test was carried out for quantification of protein concentrations. The protein (30 μ g/lane) was separated with 10% SDS-PAGE gel and transferred onto the PVDF membrane. The membrane was sealed with 5% skimmed milk for 2 hours at room temperature. The protein bands were visualized with BeyoECL Star detection kit (Beyotime, China) and quantified with the ImageJ software v1.8.0 (National Institutes of Health). β -Actin was used as loading control.

2.8 Statistical Analysis

All experiments were performed in triplicate. Results are presented as the mean $\pm$ standard deviation (SED). For the statistical analysis, GraphPad Prism 8.0 (GraphPad Software Inc.) was used to evaluate one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Genipin Inhibited Viability of Prostate Cancer Cells

To evaluate the effect of Genipin on prostate cancer cells, DU145, PC-3 and RM-1 cells were treated with different concentrations of Genipin and cell viability was determined (Figure 1 D-F). The results demonstrated that Genipin was able to disrupt the viability in both DU145, PC-3 and RM-1 cells in a dose- and time-dependent manner. Furthermore, the IC_{50} values of DU-145, PC-3 and DU-145 were calculated (Figure 1B). With an IC_{50} value of 152 μ M at 24 h, 87.97 μ M at 48 h, and 51.98 μ M at 72 h in DU145 cells, 275.8 μ M at 24 h, 198.9 μ M at 48 h, and 32.3 μ M at 72 h in PC-3 cells and 128.2 μ M at 24 h, 87.82 μ M at 48 h, and 50.19 μ M at 72 h in RM-1 cells. Based on IC_{50} calculations, further experiments were performed at concentrations of 0, 10, 50, 75, 100, 150 and 300 μ M Genipin for 24 h. In addition, Genipin had little effect on normal prostate cell WPMY1 (Figure 1C) which shows Genipin may have few toxic to normal prostate.

3.2 Genipin Suppressed the proliferation on Prostate Cancer Cells

It was found that the concentration dependence of Genipin inhibited the growth of DU145 cells (Figure 2 A). When Genipin was more than 50 μ M, the rate of colony formation decreased obviously. Furthermore, the expression of ki-67 (Proliferation Marker) and Survivin (Apoptosis Marker) was detected by Western blotting. As illustrated in (Figure 3 A), ki-67 and Survivin were reduced in DU14 cells after treated with different concentration of Genipin. In parallel, the outcome for PC-3 and RM-1 cells was similar to that of DU145 (Figure 2 B-C) & (Figure 3 B-C). Thus, the above data indicate that Genipin inhibits the proliferation of prostate cells. And the results indicated that Genipin showed dose-dependent inhibition of cell proliferation.

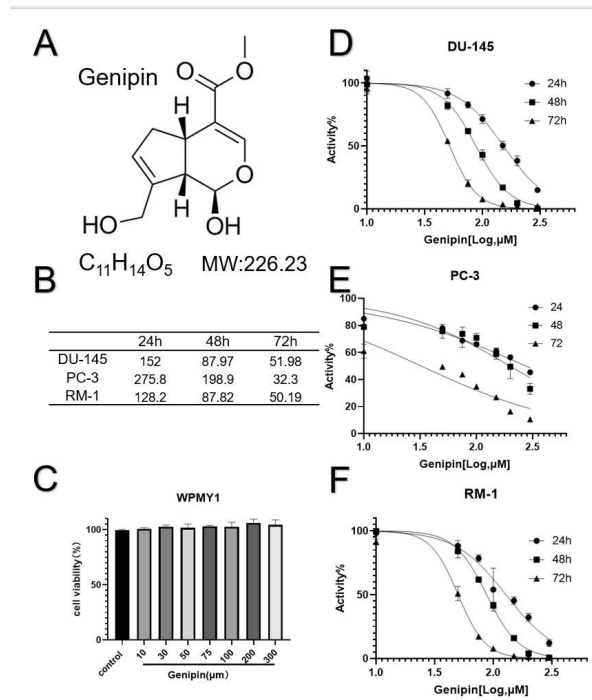


Fig. 1 Genipin Inhibited Viability of Prostate Cancer Cells. (A) The chemical structure of Genipin and its molecular weight (MW). (B) The IC_{50} value of DU-145, PC-3, RM-1 cells after treating with different concentration of Genipin. (C) CCK-8 assay were conducted to measure cell viability of normal human prostate cell WPMY1 with different concentration of Genipin (0 to 300 μ M). (D-F) CCK-8 assay were conducted to measure cell viability of human prostate cell DU145, PC-3, RM-1 with different concentration of Genipin (0 to 300 μ M).

3.3 Genipin May Inhibit DU145, PC-3 and RM-1 cells Migration

In order to assess cell migration ability, the wound-healing test was used. Genipin treatment at different concentrations (10, 30, 50 and 75 μ M) has a time dependent effect on wound closure, as shown in Figure 4 A-C. Previous studies have confirmed that the effect of Genipin on cell migration is related to the MMP₂ protein [Lee et al \(2018\)](#). Therefore, to better understand the mechanism, we used Western Blot to test the protein level of MMP₂. As shown in the Figure 5, Genipin treatment significantly inhibited the expression of MMP₂ compared with the control group.

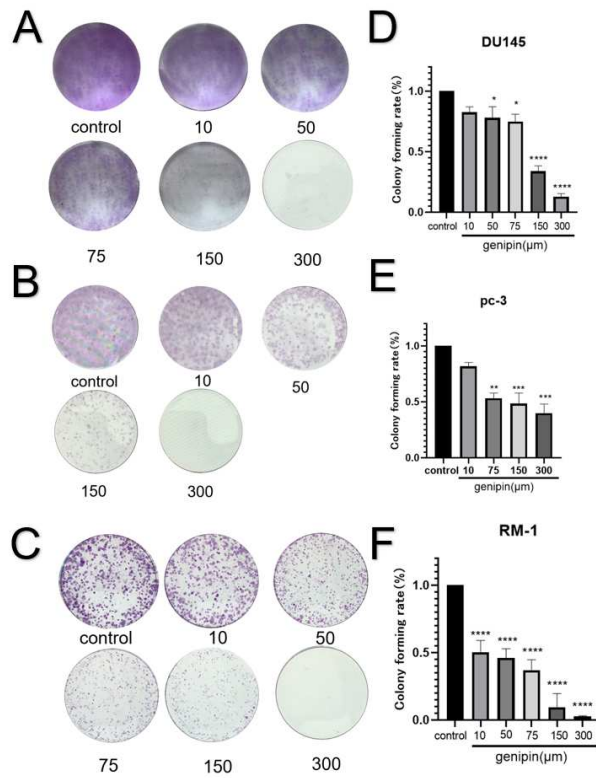


Fig. 2 Genipin suppresses the proliferation of prostate cancer cells. (A-C) DU-145, PC-3 and RM-1 cells proliferation ability was measured by clone formation assay. (D-F) The colony forming rate of DU-145, PC-3 and RM-1 cells shown as the mean $\pm$ SEM (n = 3). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 versus the control group.

3.4 Genipin Induces Mitochondrial Apoptosis in Prostate Cancer Cells

The apoptosis was characterized by Hoechst 33342/PI staining. From Figure 6, we can see that the percentage of the cells with nuclear fragmentation and condensation is significantly higher. For further research, apoptosis-related proteins were used to confirm. The tumour suppressor, p53, regulates apoptosis and repair of DNA [Gottlieb and Oren \(1998\)](#). The results showed that the expression of p53 was significantly increased by Genipin treatment (Figure 7), which suggested that p53 may play a role in the apoptosis of Genipin.

The balance between the anti-apoptotic gene BAX and the pro-apoptotic gene BCL₂ plays a key role in cellular homeostasis [Peña-Blanco and García-Sáez \(2018\)](#). Caspase-3 is regulated by a

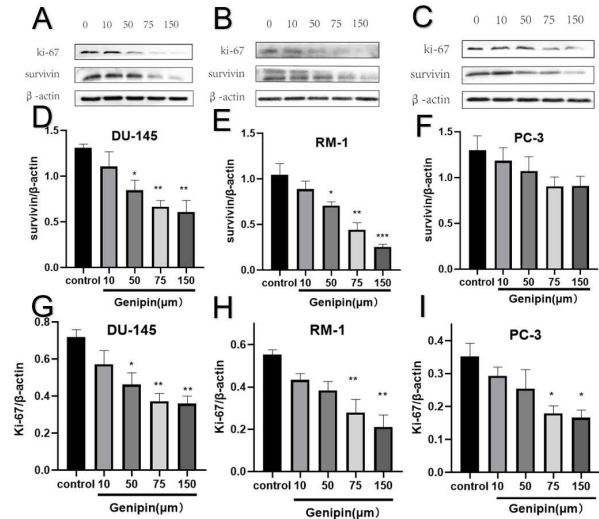


Fig. 3 Genipin suppresses the proliferation of prostate cancer cells. (A-C) After Genipin treatment (0, 10, 50, 75 and 150 μM) for 24h, the expression levels of Ki-67 and Survivin in DU-145, PC-3 and RM-1 were detected by Western blotting, respectively. β-Actin was used as the loading control. (D-F) The quantitative data of Survivin expression shown as the mean $\pm$ SEM (n = 3). *P<0.05, **P<0.01 and ***P<0.001 versus the control group. (G-I) The quantitative data of Ki-67 expression shown as the mean $\pm$ SEM (n = 3). *P<0.05 and **P<0.01 versus the control group.

number of apoptosis-related genes and is thought to be the most important terminal inhibitory enzyme in the apoptosis process; an increase in caspase-3 levels plays a key role in the initiation of apoptosis [Cohen \(1997\)](#). In addition, Bcl-2 and caspase-3 are important regulators of apoptosis induced by mitochondrial-mediated apoptosis.

In order to clarify the role of mitochondrial apoptosis in DU145, PC-3 and RM-1 cells, BCL₂, BAX and cleaved caspase-3 were evaluated by western blotting (Figure 7). The expression of pro-apoptotic BAX and cleaved caspase-3 was significantly increased after Genipin treatment, but the expression of anti-apoptotic BCL₂ was significantly reduced. Therefore, Genipin may promote cell death by activating mitochondrial pathway in prostate cells.

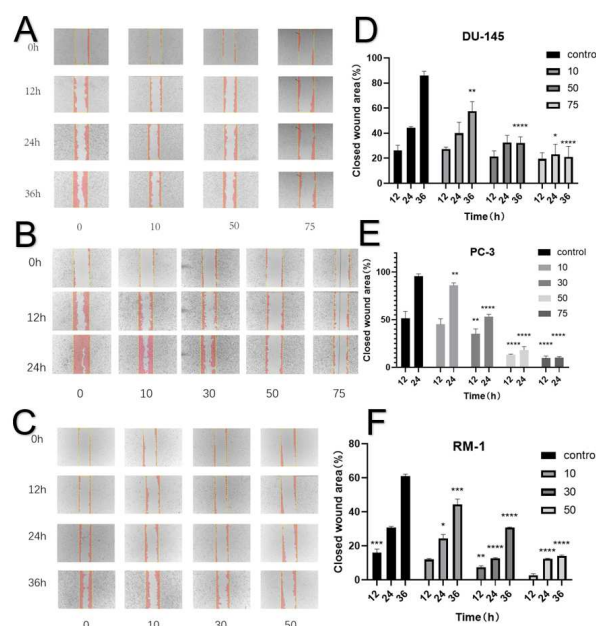


Fig. 4 Genipin may inhibit DU145, PC-3 and RM-1 cells migration. (A-C) The migratory properties of DU145, PC-3 and RM-1 cells were analyzed by wound-healing assays. Original magnification: 100x (D-F) The closed wound area of DU145, PC-3 and RM-1 cells shown as the mean $\pm$ SEM (n=3). *P<0.05, **P<0.01, ***P<0.001, **** p<0.0001 versus the control group.

3.5 Genipin Exerts Anti-prostate Cancer Effects through PI3K/AKT/NF- κ B Signaling Pathway

The activation of PI3K/AKT/NF- κ B signaling pathway was confirmed to be closely related to pathogenesis of prostate cancer [Chen et al \(2016\)](#). In addition, previous studies demonstrated that Genipin attenuates mitochondrial-dependent apoptosis through PI3K/AKT pathways [Luo et al \(2019\)](#), showing that Genipin is a potential PI3K and AKT activity in prostate cancer cells. Western blot demonstrated that Genipin inhibits PI3K/AKT/NF- κ B signaling pathway. As illustrated in Figure8, p-PI3K/PI3K, p-AKT/AKT, p-NF- κ B/NF- κ B were all reduced after Genipin treatment. suggesting that the inhibition of PI3K/AKT/NF κ B signaling by Genipin might lead to inhibition of proliferation and migration.

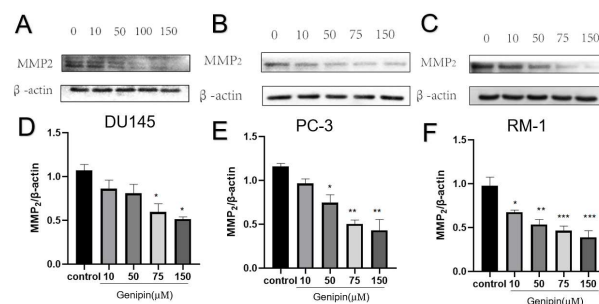


Fig. 5 Genipin may inhibit DU145, PC-3 and RM-1 cells migration. (A-C)After Genipin treatment (0, 10, 50, 75 and 150 μ M) for 24h, the expression levels of MMP₂ were detected by Western blotting. β -Actin was used as the loading control. (D-F) The quantitative data of relative protein expression shown as the mean $\pm$ SEM (n=3). *P<0.05, **P<0.01, ***P<0.001 versus the control group.

3.6 Genipin Promoted prostate cancer cells Autophagy through PI3K/AKT/mTOR Signaling Pathway

To investigate the effect of Genipin on autophagy, we studied the expression of autophagy related proteins in DU145, PC-3 and RM-1 by Western blotting. It was found that Genipin significantly reduced the expression of p62 and elevated the expression of Beclin1, LC3II/LC3I (Figure9). In addition, an autophagy inhibitor chloroquine confirmed the autophagy process. We found that chloroquine significantly effected the protein level of p62, Beclin1 and LC3II/LC3I when combined with Genipin and chloroquine (Figure9).

Since the PI3K/AKT/mTOR signaling pathway is highly prevalent in human cancer and plays an important role in the regulation of cell cycle, proliferation, apoptosis and autophagy. In order to elucidate the role of the PI3K/Akt/mTOR pathway in Genipin induction and autophagy, PI3K, AKT and mTOR were examined by Western blot with phosphorylated antibodies. As can be seen in Figure10, the protein level of phosphorylated PI3K, AKT and mTOR obviously decreased after Genipin treatment. These results demonstrated that Genipin may induce autophagy through the PI3K/AKT/mTOR Signaling Pathway in prostate cancer.

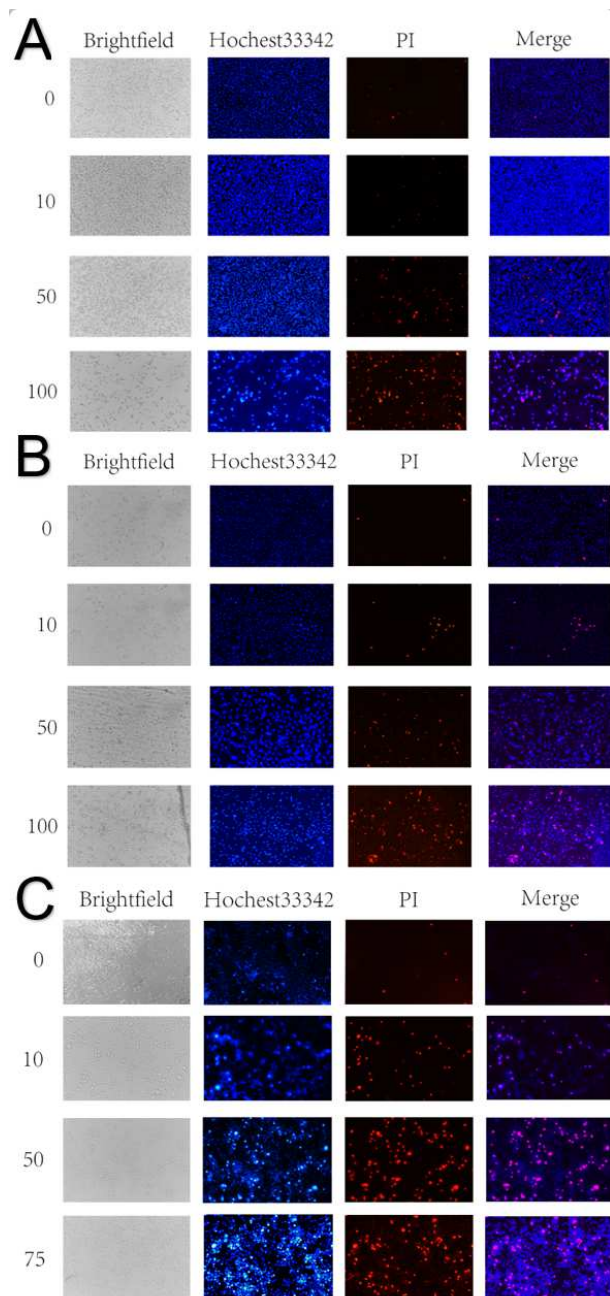


Fig. 6 Genipin Induces Mitochondrial Apoptosis in Prostate Cancer Cells. (A-C) Apoptotic morphological changes observed by Hoechst 33342/PI staining assay after Genipin treatment (0, 10, 50, 75 and 100 μ M) for 24h. Original magnification: 200x; scale bar: 20 μ m.

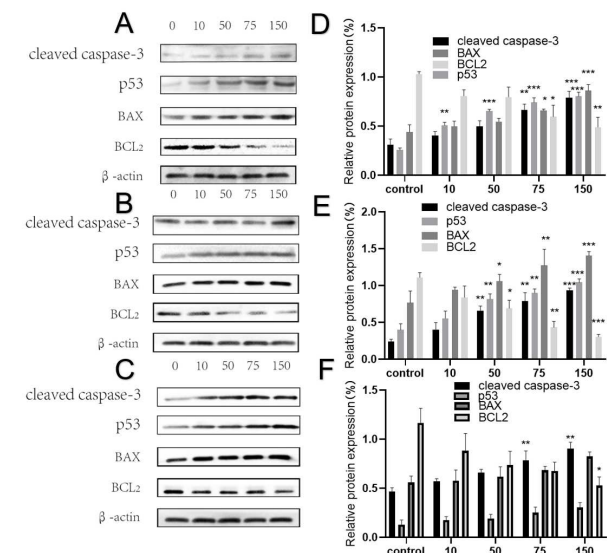


Fig. 7 Genipin Induces Mitochondrial Apoptosis in Prostate Cancer Cells. (A-C) After Genipin treatment (0, 10, 50, 75 and 150 μ M) for 24h, the expression levels of cleaved caspase-3, p53, BAX and BCL2 were detected by Western blotting. β -Actin was used as the loading control. (D-F) The quantitative data of relative protein expression shown as the mean $\pm$ SEM (n=3). *P<0.05, **P<0.01, ***P<0.001 versus the control group.

4 Discussion

Chinese medicine is one of the oldest healing systems. Nowadays, Chinese medicine has been widely used in clinical practice, which has shown great potential to prevent and treat cancer and other diseases, especially in Asia [Tariq et al \(2017\)](#). Extraction and purification of monomers from Chinese medicine is an important method for the study of traditional Chinese medicine. A number of well-known new drugs, have been successfully developed from this method are widely, such as artemisinin, an anti-malarial drug, which extract from the herb Qinghao [Tu \(2016\)](#). And the first natural anti-tumor small molecule was vinblastine [Wang et al \(2023\)](#), which then began to be found in more potent small molecules, such as paclitaxel [Rowinsky and Donehower \(1995\)](#). Facts have proved that most small molecule anticancer drugs are extracted and separated from natural substances, and the research on extraction and separation of natural substances is considered to have wider research prospects [Newman and Cragg \(2020\)](#). Gardenia jasminoides is the dry ripe fruit of gardenia of Rubiaceae. It was first found in

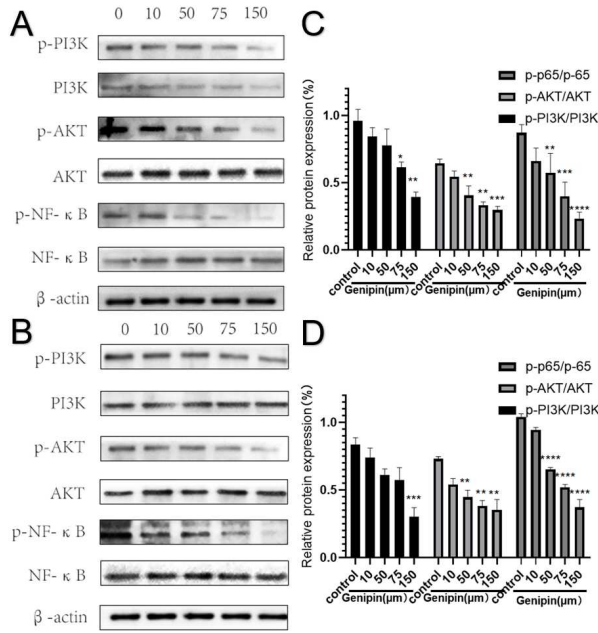


Fig. 8 Genipin Exerts Anti-prostate Cancer Effects through PI3K/AKT/NF- κ B Signaling Pathway. (A-B) After Genipin treatment (0, 10, 50, 75 and 150 μ M) for 24h, the expression levels of p-PI3K, PI3K, p-AKT, AKT, p-NF- κ B and NF- κ B were detected by Western blotting. β -Actin was used as the loading control. (C-D) The quantitative data of relative protein expression shown as the mean $\pm$ SEM (n=3). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 versus the control group.

Shen Nong Ben Cao Jing. Modern pharmacological research shows that *Gardenia jasminoides* Ellis has the effects of clearing away heat and detoxification, protecting liver [Zhong et al \(2018\)](#) and cholagogue [Liu et al \(2024\)](#), anti-inflammation [Chen et al \(2022\)](#), etc. Genipin is an active ingredient isolated from the fruit of *Gardenia jasminoides* and has a therapeutic effect in vivo. Genipin is a glycoside derived from iridoid glycoside (geniripenoid glycoside), which is converted to Genipin by the action of beta-D-glycosidase produced by intestinal flora before entering the bloodstream [Yim et al \(2004\)](#) [Yao et al \(2015\)](#).

Previous studies have shown that Genipin may inhibit the proliferation of PC-3 cells through UCP₂ protein [Yao et al \(2015\)](#), but the role of Genipin in prostate cancer remains unclear. Therefore, we first confirmed the inhibition of Genipin on prostate cancer cells DU145, PC-3, RM-1 by CCK-8 assay, and this inhibition

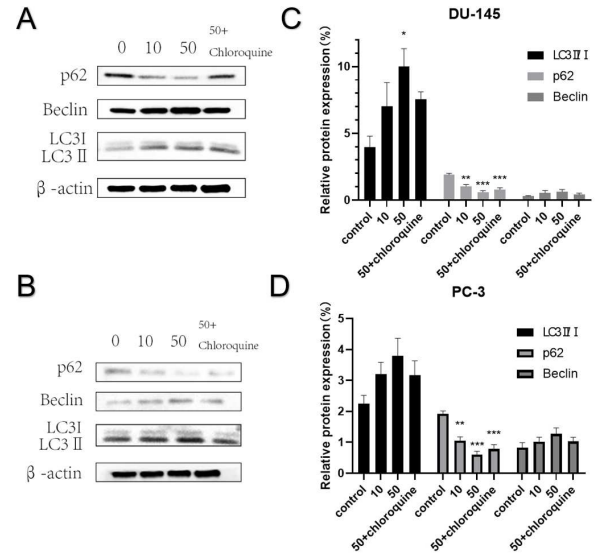


Fig. 9 Genipin Promoted prostate cancer cells Autophagy through PI3K/AKT/mTOR Signaling Pathway. (A-B) After Genipin treatment (0, 10, 50 and 50 μ M+Chloroquine) for 24h, the expression levels of p62, Beclin and LC3II/LC3I were detected by Western blotting. β -Actin was used as the loading control. (C-D) The quantitative data of relative protein expression shown as the mean $\pm$ SEM (n=3). *P<0.05, **P<0.01, ***P<0.001 versus the control group.

increased with time and concentration. In addition, treatment with different concentrations of Genipin on normal prostate cell WPMY1 showed that Genipin was almost non-toxic to normal prostate cancer cells. We speculate that Genipin may be less toxic to the human prostate. But more in vivo experiments are needed to prove it. Secondly, colony formation experiments showed that Genipin suppressed proliferation on Prostate Cancer Cells, which proved by protein ki-67 and survivin decreased with Genipin use. Studies have shown that Genipin can inhibit the invasion and migration of colorectal cancer by inhibiting HIF-1A accumulation and VEGF expression [Lee et al \(2018\)](#). We demonstrated that Genipin can inhibit the migration of DU145, PC-3 and RM-1 cells, which may be related to the migration related protein MMP₂.

Studies have shown that Genipin has anti-inflammatory [Li et al \(2022\)](#), antiangiogenic ?, antioxidant [Koriyama et al \(2010\)](#), and anti-insulin resistance [Guan et al \(2013\)](#) effects and is used to treat a variety of inflammatory diseases,

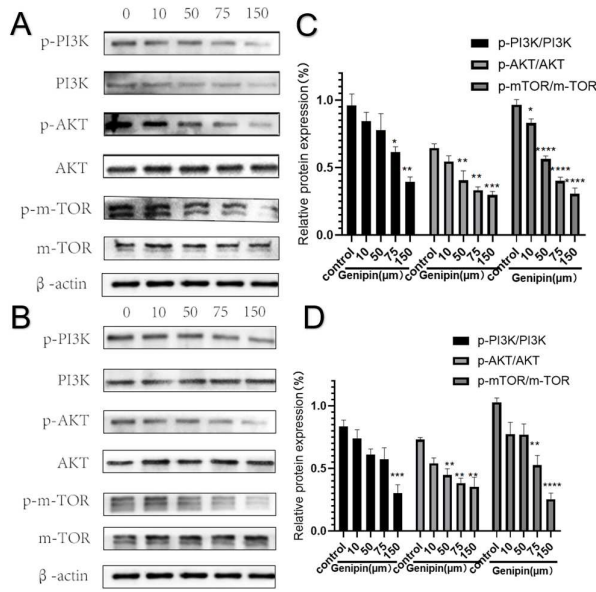


Fig. 10 Genipin Promoted prostate cancer cells Autophagy through PI3K/AKT/mTOR Signaling Pathway. (A-B) After Genipin treatment (0, 10, 50, 75 and 150 μ M) for 24h, the expression levels of p-PI3K, PI3K, p-AKT, AKT, p-m-TOR and m-TOR were detected by Western blotting. β -Actin was used as the loading control. (C-D) The quantitative data of relative protein expression shown as the mean $\pm$ SEM (n=3). *P<0.05, **P<0.01, ***P<0.001, **** p<0.0001 versus the control group.

including cancer Yang et al (2013). It has been reported that the antitumor effects of Genipin may be mediated by various mechanisms, including apoptosis Xia et al (2021), stopping of cell cycle Bai et al (2022), inhibiting invasion and metastasis Lee et al (2018). A variety of signaling pathways, including mTOR, STAT3, BAX and BCL₂ are involved in the apoptosis of cancer cells. Apoptosis is a form of programmed cell death that results in the orderly and efficient removal of damaged cells Pistritto et al (2016). And previous studies have shown that, Genipin signaling to apoptosis of PC-3 cells is mediated via activation of (ROS)-MLK3-c-Jun NH2-terminal kinase (JNK)-dependent activation of mitochondrial pathway Hong and Kim (2007). Genipin induces p53 and BAX-dependent apoptosis in colon cancer cells Ye et al (2018). In this study, we demonstrated that Genipin causes apoptosis of prostate cancer cells by fluorescent staining. The expression of apoptosis-related proteins was confirmed by Western Blot assay.

It has been shown that Genipin induced PI3K/AKT signaling pathway leads to inhibit the growth in osteosarcoma Huang et al (2023) and human bladder cancer Li et al (2018). At present, The PI3K/Akt pathway plays an important role in the development and survival of cancer cells. Their clinical efficacy is limited, however, as the PI3K family is activated by a complex mechanism Mishra et al (2021). Therefore, the combination of PI3K/AKT inhibitors with other anticancer therapies has been suggested to address the treatment dilemma Vara et al (2004). NF- κ B, a transcription factor involved in the development and progression of cancer, is one of the downstream pathways of AKT Sruthi et al (2024). Therefore, inhibition of the PI3K/AKT/NF- κ B pathway may be related to the anti-prostate cancer activity of Genipin, which may be a promising lead agent in prostate cancer therapy. In our research, the inhibition of PI3K/AKT/NF- κ B has been demonstrated by Western blot. However, Chinese medicine is characterized by multi-composition, multi-target and synergic effect. The present study provides a case study on the pharmacological mechanism of Chinese medicine. Nevertheless, further confirmation of the inhibition of tumor growth and metastasis by the PI3K/AKT/NF- κ B pathway is required.

Autophagy is the catabolism of its components by the lysosomal machinery, which plays a crucial role in the survival of cells and crucial for homeostasis, development, and other pathophysiological processes Debnath et al (2023). In addition, autophagy is associated with LC3II/I, Beclin-1, and ATG5. In this study, we first studied the effect of Genipin on the autophagy of prostate cancer. We found that Genipin could increase Beclin1 and LC3II/I protein levels, but decrease P62 protein level. The autophagy process was relieved after co-incubation with autophagy inhibitor chloroquine, as compared with Genipin alone. Previous research demonstrate that the antiapoptotic BCL₂ family members not only relate to apoptosis inhibition, instead affect autophagy indirectly, but also owing to their inhibition of BAX and Bak Lindqvist and Vaux (2014). Therefore, in combination with up-regulation of BAX and down-regulation of BCL2 after Genipin treatment, we proposed that Genipin could induce autophagy in prostate cancer.

It is well known that PI3K/AKT/mTOR pathway plays an important role in the progression and carcinogenesis of various cancers. And the PI3K/Akt/mTOR pathway may be a promising therapeutic target for prostate cancer. Previous studies have shown that Genipin passes inhibition of UCP₂ triggers Akt/mTOR pathway in a ROS-dependent mechanism in pancreatic adenocarcinoma cells [Dando et al \(2017\)](#). In addition, some studies have demonstrated that Genipin induces autophagy through PI3K/AKT/mTOR pathway and suppresses cell growth in oral squamous cell carcinoma [Wei et al \(2020\)](#). In this study, we found that Genipin inhibited PI3K, AKT and mTOR phosphorylation in a concentration-dependent manner, suggesting the inactivation of PI3K/Akt/mTOR pathway. Thus, Genipin is thought to inhibit the growth of prostate cells and improve autophagy by inhibiting the PI3K/Akt/mTOR signaling pathway.

However, the poor bioavailability and potential toxicity limit the clinical application of Genipin. Genipin is the main hepatotoxic substance of gardenia [Z et al \(2023\)](#). Moreover, the safety concern exists as the precise target of Genipin is unknown and the excessive inhibition of PI3K/AKT/NF- κ B pathway may bring about side effects. Hence, Genipin treatment may be more potent but not necessarily more effective than existing treatment options, which needs further evaluation. But new research suggests that Genipin's ring-opening tautomerism may be an important material underlying hepatotoxicity [C et al \(2023\)](#). In the meantime, a number of novel drug delivery systems have been designed to improve the bioavailability and minimize side effects of low-solubility natural medicines [Yang et al \(2020\)](#). Further research aiming to enhance the anticancer effects of Genipin would prove beneficial. Future studies may focus on combining Genipin with other cancer treatments to increase the therapeutic effect.

5 Conclusion

In conclusion, this study demonstrated that Genipin, the active compound of Gardenia fruits, might inhibit proliferation and migration of prostate cancer through PI3K/AKT/NF- κ B signaling pathway and promoted prostate cancer cells autophagy through PI3K/AKT/mTOR signaling pathway. This study provides a rationale

of using Genipin as the potential lead drug for prostate cancer treatment.

Declarations

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- Conflict of interest/Competing interests The authors have no relevant financial or non-financial interests to disclose.
- Consent for publication Not applicable
- Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.
- Materials availability
- Code availability
- Author contribution

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